The effect of Paf antagonists on bronchial hyperresponsiveness induced by Paf, propranolol or indomethacin

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- 1 Intravenous administration of platelet activating factor, Paf ($600 \text{ ng kg}^{-1} \text{ h}^{-1}$) to ventilated anaesthetised guinea-pigs induced bronchial hyperresponsiveness to i.v. acetylcholine.
- 2 Pretreatment of ventilated, anaesthetised guinea-pigs with the β -adrenoceptor antagonist, propranolol, or the non-steroidal anti-inflammatory drug, indomethacin, induced bronchial hyperresponsiveness to i.v. histamine.
- 3 Paf-induced bronchial hyperresponsiveness was significantly attenuated by pretreatment with three different Paf antagonists, CV-3988, BN 52021 and WEB 2086.
- 4 Pretreatment of guinea-pigs with CV-3988, BN 52021 or WEB 2086 at doses inhibiting Paf-induced bronchial hyperresponsiveness, had no significant effect on propranolol or indomethacin-induced bronchial hyperresponsiveness.
- 5 It is suggested that bronchial hyperresponsiveness induced by propranolol or indomethacin is not secondary to Paf release in the guinea-pig.

Introduction

Increased airway responsiveness to a wide range of stimuli is one of the characteristic features of bronchial asthma (Hargreave *et al.*, 1986). Exposure to allergens, viral infections, oxidant gases such as ozone or nitrogen dioxide, or sensitising chemicals such as toluene diisocyanate can induce airway hyperresponsiveness in both man and experimental animals (Barnes, 1986). However, it is also recognised that certain classes of drugs administered to asthmatics will also induce bronchoconstriction and increase the responsiveness of the airways.

Shortly after their introduction into clinical use, β -adrenoceptor antagonists such as propranolol were found to cause bronchospasm (McNeil, 1964; McNeil & Ingram, 1966), an effect which also occurred in the guinea-pig (MacLagan & Ney, 1979). This was assumed to be a consequence of β -adrenoceptor blockade in the lungs, but even the use

of cardioselective β -adrenoceptor antagonists did not reduce the incidence of bronchospasm (Skinner *et al.*, 1975). Additionally, β -adrenoceptor antagonists have been found to increase the responsiveness of the airways to other bronchoconstrictor agents such as histamine and acetylcholine (Ney, 1983).

Another class of drugs known to induce bronchoconstriction in a subset of asthmatics and to increase the responsiveness of the airways to standard spasmogens are the non-steroidal anti-inflammatory drugs (NSAIDs) (Adcock & Garland, 1980; Mitchell, 1983; 1988; Mitchell & Adcock, 1988). However, the mechanisms underlying this drug-induced bronchial hyperresponsiveness remain to be elucidated.

Recent evidence has suggested that an ether-linked phospholipid, platelet activating factor (Paf), is able to induce a long lasting, non-specific increase in bronchial responsiveness in experimental animals (Mazzoni *et al.*, 1985; Chung *et al.*, 1986; Christman *et al.*, 1987; Barnes *et al.*, 1987; Robertson & Page,

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1987; Coyle *et al.*, 1988; Robertson *et al.*, 1988) and man (Cuss *et al.*, 1986). We have, therefore, investigated whether bronchial hyperresponsiveness induced by either indomethacin or propranolol is due to the release of Paf by the use of three distinct classes of Paf antagonists: a Paf analogue (CV-3988), a ginkgolide (BN 52021) and a triazolo-benzodiazepine (WEB 2086).

Methods

Male Dunkin-Hartley guinea pigs (300–600 g) were anaesthetised with urethane (25% w/v, 7 ml kg⁻¹; i.p.) and the trachea, carotid artery and jugular vein cannulated for measurement of airway obstruction, systemic blood pressure and drug administration respectively. Guinea-pigs were ventilated with room air at 70 strokes min⁻¹ with a stroke volume of 1 ml 100 g⁻¹ body weight. Airway obstruction was measured using a differential pressure transducer connected to the side-arm of the tracheal cannula, and pulmonary inflation pressure (PIP) measured in mmHg.

Specificity of Paf antagonists

Acetylcholine (ACh) (5 µg kg⁻¹) or histamine (5 µg kg⁻¹) were administered intravenously as a bolus before and 30 min after treatment with BN 52021 (20 mg kg⁻¹, i.v.), CV-3988 (1 mg kg⁻¹ i.v.) or WEB 2086 (1 mg kg⁻¹ i.v.).

Airway hyperresponsiveness

Induced by Paf ACh (5 µg kg⁻¹) was administered intravenously as a bolus before, and 10 min after the infusion of Paf (600 ng kg⁻¹ over 1 h). The total dose of Paf was infused at three increasing rates, (5% in 10 min, 20% in 20 min and 75% in 30 min) as described previously (Robertson & Page, 1987). The percentage increase in PIP induced by ACh (5 µg kg⁻¹, i.v.) was calculated before and after the infusion of Paf. Hyperresponsiveness was assessed by the incremental increase in response to ACh, determined by calculating the difference between the increase in PIP (%) induced by ACh before and after the infusion of Paf. Bronchial hyperresponsiveness was measured in animals pretreated with one of the various Paf antagonists or the appropriate vehicle control, according to the following protocol: WEB 2086 (1 mg kg⁻¹) or vehicle administered i.p. 1 h before anaesthesia; CV 3988 (1 mg kg⁻¹) or vehicle

administered i.v. immediately before infusion of Paf; BN 52021 (20 mg kg⁻¹) or vehicle administered i.v. 30 min before anaesthesia.

Induced by propranolol or indomethacin Bronchoconstriction was induced by histamine (1–64 µg kg⁻¹, i.v.) and the dose required for a 50–100% increase in PIP was selected. This dose of histamine (2–8 µg kg⁻¹) was then given before and 15, 30 and 60 min after either propranolol (0.1 mg kg⁻¹, i.v.) or indomethacin (5 mg kg⁻¹, i.v.). Paf antagonists were administered according to the following protocol: WEB 2086 (1 mg kg⁻¹, i.p.) 1 h before propranolol or indomethacin; CV 3988 (1 mg kg⁻¹, i.v.) 10 min before propranolol or indomethacin, and BN 52021 (20 mg kg⁻¹, i.p.) 30 min before propranolol or indomethacin.

Results are expressed as the percentage increase in the PIP response to histamine at each time interval after treatment with either indomethacin or propranolol.

Materials

Acetylcholine chloride, bovine serum albumin (BSA; fraction V, essentially fatty acid free) and urethane were purchased from Sigma and dissolved in physiological saline. Paf was obtained from Novabiochem Ltd and prepared in 0.25% BSA. (±)-Propranolol hydrochloride (Imperial Chemical Industries plc) was dissolved in physiological saline and indomethacin (Sigma) was dissolved in Na₂CO₃. WEB 2086 (3-[4-(2-chlorophenyl)-9-methyl-6H-thieno[3,2-f][1,2,4]triazolo-[1,4]-diazepin-2-yl]-1-(4-morpholinyl)-1-propanone) (a gift from Dr Heuer, Boehringer Ingelheim KG, West Germany) was dissolved in saline, CV 3988 ((RS)-2-methoxy-3-(octadecylcarbamoyloxy)propyl-2-(3-thiazolo) ethyl phosphate) (a gift from Takeda Chemical Industries Ltd) was dissolved in heated saline and the pH adjusted to 7 by adding 1 M NaOH, BN 52021 ([3(1,1-dimethylethyl)hexahydro-1,4,4,7-B-trihydroxy-8-methyl 9H-1,7-(epoxymethanol) - 14,6 - alpha - H₂ - cyclopenta(c)furo[2,3b]furo[3',2',3,4] cyclo-penta [1,2-d]furan-5,9,12 (4H)-trione) (a gift from Dr P. Braquet, Institute Henri Beaufour, Le Plessis Robinson, France) was prepared as a suspension in acacia gum (10%) for i.p. administration and in 2% DMSO for i.v. administration.

Statistical analysis

Data are presented as the mean ± s.e.mean of *n* observations. Significance was assessed by the non-

Table 1 Specificity of Paf antagonists against histamine- and acetylcholine (ACh)-induced bronchoconstriction

		BN 52021 (20 mg kg ⁻¹ i.v. bolus)	WEB 2086 (1 mg kg ⁻¹ i.v. bolus)	CV-3988 (1 mg kg ⁻¹ i.v. bolus)
ACh (5 µg kg ⁻¹ i.v.)	Before drug	56.25 ± 3.8	49.3 ± 8.7	47.9 ± 6.02
	After drug	52.0 ± 9.6	49.2 ± 8.1	37.02 ± 3.3
Histamine (5 µg kg ⁻¹ i.v.)	Before drug	146.0 ± 18.6	151.2 ± 26.5	141.4 ± 29.71
	After drug	137.0 ± 22.7	152.5 ± 22.0	135.8 ± 26.7

The effect of pretreatment with Paf antagonists on % increase in pulmonary inflation pressure (PIP) induced by ACh or histamine. Results shown are mean ± s.e. % increase in PIP before and after Paf antagonists, $n = 5-7$ animals. None of the Paf antagonists utilised had any significant effect on ACh or histamine-induced increases in PIP.

parametric Wilcoxon matched-pairs signed-ranks test, with $P < 0.05$ being considered significant.

Results

The intravenous bolus administration of ACh (5 µg kg⁻¹) or histamine (5 µg kg⁻¹) induced significant increases in PIP which were not modified by any of the Paf antagonists utilised (Table 1).

The intravenous infusion of Paf in control guinea-pigs (treated with appropriate vehicles for antagonists) induced an increase in the airway responsiveness to ACh. This increase was significantly reduced by pretreatment with each of the Paf antagonists used (Figures 1-3).

Propranolol (0.1 mg kg⁻¹ i.v.) and indomethacin (5 mg kg⁻¹ i.v.) induced an increase in the airway responsiveness to histamine (Figure 4 and Table 2), and this effect was maximal at 30 min after the administration of each compound.

Figure 1 Airway responsiveness to acetylcholine (ACh, 5 µg kg⁻¹, i.v.) before (open columns) and after (hatched columns) an infusion of Paf (600 ng kg⁻¹ h⁻¹, i.v.) in vehicle or CV-3988 (1 mg kg⁻¹, i.v.) treated animals. Results shown are the mean percentage increase in pulmonary inflation pressure (PIP) in 5-11 separate experiments and vertical lines show s.e.mean. Responses to ACh were significantly increased after Paf (* $P < 0.001$), but no significant increase was observed in animals pretreated with CV-3988 (NS).

Figure 2 Airway responsiveness to acetylcholine (ACh, 5 µg kg⁻¹, i.v.) before (open columns) and after (hatched columns) an infusion of Paf (600 ng kg⁻¹ h⁻¹, i.v.) in vehicle or WEB 2086 (1 mg kg⁻¹, i.p.) treated animals. Results shown are the mean percentage increase in pulmonary inflation pressure (PIP) of 5-11 separate experiments and vertical lines show s.e.mean. Responses were significantly increased after Paf (* $P < 0.001$), but no significant increase was observed in animals pretreated with WEB 2086 (NS).

Figure 3 Airway responsiveness to acetylcholine (ACh, $5 \mu\text{g kg}^{-1}$, i.v.) before (open columns) and after (hatched columns) an infusion of Paf (600 ng kg^{-1} , i.v.) in vehicle or BN 52021 (20 mg kg^{-1} , i.v.) treated animals. Results shown are the mean percentage increase in pulmonary inflation pressure (PIP) of 4 separate experiments and vertical lines show s.e.mean. Responses were significantly increased after Paf (* $P < 0.001$), but no significant increase was observed in animals pretreated with BN 52021 (NS).

Table 2 Effect of Paf antagonists on propranolol and indomethacin-induced bronchial hyper-responsiveness

Drug	% increase PIP	
	Histamine $5 \mu\text{g kg}^{-1}$	Histamine $5 \mu\text{g kg}^{-1}$ after propranolol 0.1 mg kg^{-1} i.v.
Saline	110 ± 3	263 ± 40
BN 52021	92 ± 2	259 ± 14
20 mg kg^{-1} i.v.		
WEB 2086	96 ± 1	233 ± 26
1 mg kg^{-1} i.v.		
CV 3988	120 ± 2	264 ± 33
1 mg kg^{-1} i.v.		
	% increase PIP	
	Histamine $5 \mu\text{g kg}^{-1}$	Histamine $5 \mu\text{g kg}^{-1}$ after indomethacin 1 mg kg^{-1} i.v.
Saline	85 ± 1	274 ± 56
BN 52021	79 ± 2	230 ± 56
20 mg kg^{-1} i.v.		
WEB 2086	90 ± 2	220 ± 30
1 mg kg^{-1} i.v.		
CV 3988	80 ± 2	222 ± 52
1 mg kg^{-1} i.v.		

The effect of pretreatment with Paf antagonists or saline on % increase in pulmonary inflation pressure (PIP) induced by intravenous histamine ($5 \mu\text{g kg}^{-1}$; $n = 5$) before and after treatment with propranolol (0.1 mg kg^{-1} i.v.) or indomethacin (5 mg kg^{-1} i.v.).

Figure 4 Percentage increase in pulmonary inflation pressure (PIP) induced by intravenous histamine before and at intervals after pretreatment with either propranolol (●) or indomethacin (○). Results are expressed as mean of 4–6 experiments and vertical lines indicate s.e.mean.

CV 3988, WEB 2086 and BN 52021 were all without significant effect on the airway hyper-responsiveness induced by propranolol and indomethacin (Table 2).

Discussion

Indomethacin has been shown to enhance the airway responsiveness to the spasmogen histamine both *in vitro* (Adcock & Garland, 1980) and *in vivo* (Mitchell, 1983; 1988). This phenomenon is not peculiar to indomethacin since other NSAIDs such as flufenamate, aspirin and phenylbutazone have also been shown to increase the bronchoconstrictor response to histamine in guinea-pigs (Mitchell & Adcock, 1987). Recent evidence has suggested that this phenomenon may involve an action of NSAIDs on the vagus nerve (Mitchell, 1988). In about 10% of patients with asthma, aspirin and other NSAIDs precipitate asthmatic attacks. Recently, platelets taken from aspirin-sensitive asthmatics have been demonstrated to release cytotoxic oxygen-free radicals when incubated with aspirin *in vitro*, whilst sodium salicylate, which is structurally related to aspirin but does not inhibit cyclo-oxygenase, does not activate such platelets (Ameisen *et al.*, 1985). These and other observations implicate the participation of platelets in the aetiology of this condition (Page, 1988a). Recently Schmitz-Schumann *et al.* (1987) have demonstrated that Paf release accompanies bronchoconstriction induced by NSAIDs in

aspirin-sensitive asthmatics. As Paf is able to induce bronchial hyperresponsiveness (Mazzoni *et al.*, 1985; Chung *et al.*, 1986; Barnes *et al.*, 1987; Robertson & Page, 1987; Robertson *et al.*, 1988; and present study), it was possible, therefore, that release of Paf could contribute to airway hyperresponsiveness induced by NSAIDs, particularly since Paf-induced bronchial hyperresponsiveness in the guinea-pig is known to be dependent on platelet activation (Mazzoni *et al.*, 1985).

However, the present results show that none of the Paf antagonists tested had any significant effect upon indomethacin-induced airway hyperresponsiveness in the guinea-pig, at concentrations that clearly inhibit Paf-induced bronchial hyperresponsiveness. The doses used to inhibit Paf-induced bronchial hyperresponsiveness were also comparable to those found previously to inhibit other biological actions of Paf (e.g. bronchoconstriction or hypotension) (Terashita *et al.*, 1983; Braquet *et al.*, 1985; Casals-Stenzel *et al.*, 1987) and our results indicate that at the concentrations employed, BN 52021, WEB 2086 and CV-3988 do not have muscarinic or H₁-receptor antagonist activity. These results suggest, therefore, that Paf does not play a central role in bronchial hyperresponsiveness induced by NSAIDs in the guinea-pig.

The mechanism of airway hyperresponsiveness induced by β -adrenoceptor antagonists is completely unknown. Ney (1983) demonstrated that it was not due to β -adrenoceptor blockade, since the dextro-rotatory isomer of propranolol and the racemic mixture were equipotent at producing airway hyperresponsiveness, whilst the dextrorotatory isomer is

considerably less potent as a β -adrenoceptor antagonist. Interestingly, the β -adrenoceptor agonist isoprenaline has also been demonstrated to induce airway hyperresponsiveness in the guinea-pig, this effect being additive with propranolol (Mazzoni *et al.*, 1987) and unrelated to occupancy of β -adrenoceptors. Ney (1983) showed that part of the propranolol-induced hyperresponsiveness in guinea-pigs could be inhibited by the leukotriene antagonist FPL-55712 or the combined lipoxygenase/cyclooxygenase inhibitor BW755C, which suggests a role for lipoxygenase-derived mediators in the pathogenesis of part of this phenomenon. We considered that Paf may also play a role in this phenomenon as it is often released from membrane phospholipids alongside arachidonic acid metabolites and is able to induce bronchial hyperresponsiveness. However, the present study clearly demonstrated that none of the Paf antagonists was able to inhibit significantly propranolol-induced bronchial hyperresponsiveness at doses that clearly inhibited Paf-induced bronchial hyperresponsiveness. Therefore, whilst Paf is able to induce many of the characteristic features of asthma, including bronchial hyperresponsiveness (Page, 1988b), our present data would suggest that this phospholipid is not involved with bronchial hyperresponsiveness induced by either NSAIDs or propranolol.

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